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Attorney Docket No. 04012.0188

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Prieels April___, 1998

Serial No.: 08/909,879 Group Art Unit No.: 1818

Filed: August 12, 1997 Examiner: L. Smith

For: VACCINE COMPOSITION CONTAINING ADJUVANTS

Assistant Commissioner of Patents

Washington, D.C. 20231

DECLARATION OF DR. GERALD VOSS

1. I, Dr. Gerald Voss, a citizen of Germany and residing at , 12 Rue de la Barre, Grez-doiceau, Belgium, state and declare the following with respect to the invention described and claimed in U.S. Patent application No. 08/909,879 (Attorney Docket No. 04012.0188), entitled "VACCINE COMPOSITION CONTAINING ADJUVANTS."

2. I have received the following academic qualifications:

- Degree in Biology '83-'85, University of Freiburg, Germany.
- Master's degree in Biology (German diploma) '89, University of Goettingen, Germany
- Ph.D. in Biology '92, University of Goettingen and German Primate Centre, Goettingen.

I undertook a postdoctoral research position in Medicine, '93-'96, Harvard Medical School, Boston, USA. I joined SmithKline Beecham Biologicals in 1996, as a research group leader, specifically as an immunologist in the HIV, malaria, Dengue and adjuvant evaluation programs.

3. I have read and am familiar with the Office Action dated November 24, 1997, issued in the above captioned action.

4. I understand that the Examiner rejected the claims directed to a vaccine composition containing HIV and FIV antigens because, according to the Examiner, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. In my opinion, and as explained in the response, the specification enables a person skilled in the art to make the HIV and FIV vaccine compositions of the subject invention and to use them as effective vaccines against HIV and FIV infections. Several studies and tests conducted by SmithKline Beecham Biologicals or under the Company's direction, which are described in the paragraphs below, support this conclusion.

The role of HIV-specific CTL in HIV infection episodes

5. In recent years much experimental work has been performed with the aim of identifying particular immunological responses which are important in the immunological control of HIV infections. In my opinion recent published work has suggested the important role of CTL in the control of HIV infections.

6. For example, CTL's have been shown to be activated *in vivo* to high levels, which activation coincides with a reduction of viraemia (1, 2, 3). Further suggestive evidence about the important role of CTL include observations that HIV-specific CTL are crucial in the maintenance of the asymptomatic phase of infection, before the development of the acquired immune deficiency syndrome (AIDS). Thus long-term non-progressors have high levels of HIV specific CTL, whereas HIV specific CTL are lost during progression to AIDS (4, 5, 6, 7, 8, and 9).

7. CTL have been associated with virus clearance from infected individuals and also with clinical protection from a number of viral infections. Accordingly, a number of studies have suggested that vaccine-induced protection against simian and feline immunodeficiency viruses, is correlated with the induction of strong antigen-specific CTL responses (10, 11).

8. Thus, the induction of anti-HIV CTL is not merely the induction of an immune response. It is the induction of the type of immune response which is postulated to be a major correlate of HIV prophylaxis or therapy.

The choice of the experimental animal model for the investigation of putative HIV vaccines

9. During recent years a number of authors have attempted to identify the most relevant model for testing of potential prophylactic and therapeutic anti-HIV vaccines. For example, the chimpanzee has been used in a number of early HIV studies because of its susceptibility of HIV infections (17, 18). However, the observation that chimpanzees do not develop AIDS-like symptoms has led to suspicion that these studies may not be the most relevant model for investigating the immuno-prophylactic and therapeutic role of anti-HIV vaccines. The chimpanzee model has also been criticised for several other reasons including, ethical and regulatory difficulties; high costs and limited supply of chimpanzees; and also high costs of safe and sufficiently large containment facilities.

10. Currently, the best model to study anti-HIV vaccines is the simian-human immunodeficiency virus (SHIV) infection of the rhesus monkey. The chimeric SHIV expressing the HIV-1 Env protein allows for the evaluation of HIV-1 vaccines in a simple and relevant animal model (12). Infected animals mount a vigorous immune response similar to those observed in infected humans, including CD8+ CTL (13). In contrast to the HIV-1 infection of chimpanzees, infection of rhesus monkeys with some SHIV strains induces typical AIDS-like symptoms (14). The most prominent feature of this disease induction is a rapid CD4+ cell decline (15, 20). Molecular clones of the pathogenic virus have now been characterised (16).

11. The SHIV/rhesus monkey model of HIV infection provides the most feasible model at the present time for the testing of potential prophylactic and therapeutic HIV vaccines. Furthermore, the SHIV rhesus monkey model does not share the other

criticisms as those experienced with the chimpanzee model to the same extent. Rhesus monkeys are smaller and easier to handle, are more extensively available at reasonable cost.

Experimental evidence performed with vaccines of the present invention

12. A number of experiments have been carried out which investigate the combination of 3D-MPL and QS21 for use as vaccine adjuvants for the development of a human anti-HIV vaccine. Such experiments are described herein and include vaccination studies in murine and rhesus monkey models, and also phase I human trials.

The generation of HIV-specific CTL in the murine model and in human vaccine trials.

13. The generation of HIV-specific CD8+ CTL is thought to be crucial for any successful anti-HIV vaccine (see above). The following experiments describe the induction of potent anti-HIV CTL responses in the murine model. Thus, vaccine adjuvants of the present invention, comprising 3D-MPL and QS21, are capable of stimulating one type of immune responses which have been postulated to be crucial for any successful anti-HIV vaccine.

14. Groups of 8 mice were vaccinated on two occasions with the vaccine formulations as described in table 1. The results shown in table 2, and figures 1, and 2, demonstrate that strong anti-HIV humoral and CTL responses were induced with the 3D-MPL/QS21 adjuvants. These results are summarised in table 3.

15. A phase I study, to investigate human application of an HIV vaccine, has been performed. Such trials are primarily designed for the purpose of measuring the safety, reactogenicity, and volunteer tolerance of the vaccine candidate. The phase I trial was successful in that it demonstrated the immunogenicity and safety of such an HIV vaccine. Work is now continuing in this area with the aim of future clinical studies.

16. During the phase I clinical study, the opportunity arose to make initial, preliminary, attempts to detect HIV-specific CTL in the human vaccinated volunteers. The detection of CTL, which was not the aim of the study, was unsuccessful, but was attempted because of the unique opportunity of having source of post HIV-vaccination human PBMC.

17. The observation that no HIV-specific CTL were detected in the PBMC at a time point 4 weeks after vaccination was not surprising. Reasons as to why circulating HIV-specific CTL were not detected include the fact that any CTL that were induced could have selectively populated a particular region of the body (*e.g.* lymph nodes) which precluded their detection in circulating PBMC. The timing of the sample schedule was not ideal for the detection of circulating CTL, as CTL may have been present at times other than this single time point 4 weeks after vaccination. Moreover, this particular attempt to detect CTL only involved a very limited number of individuals (4-5 individuals out of a group of 10 volunteers), such that the possibility of the induction of CTL in individuals vaccinated in a larger study could not be precluded. Furthermore, it was recognised that the CTL detection assay was sub-optimal due to the lack of positive control samples. Despite all of the above, as the PBMC were available at this time it was thought to be worthwhile to try to detect HIV-specific CTL.

18. Following the successful completion of the phase I trial, and the observation that the vaccine was immunogenic and well tolerated, SmithKline Beecham continues to work and invest in this area, including future clinical trials.

Experimental vaccination and challenge studies using the SHIV Rhesus monkey model.

19. In the experimental studies performed, vaccination regimes comprising 3D-MPL and QS21, and the well known and characterised $gp120_{W6.1D}$ (recombinantly produced in CHO cells using a sequence derived from the HIV strain W6.1D (19)) were successful in inducing strong *in vitro* virus neutralising humoral responses. The

vaccination regime induced protection from challenge with the homologous $SHIV_{W6.1D}$ virus, in two of the four vaccinees. This homologous virus strain initiates a clinical infection which does not progress to AIDS-like symptoms, it is therefore a valid model for investigating vaccine prophylaxis of HIV infection. All of the control animals became infected (n=4). The formulations used and the results obtained are summarised in tables 4, 5 and 6.

20. Assays designed to detect HIV-specific CTL responses did not generate any interpretable data, this was because of the lack of a fully characterised assay system with appropriate control samples.

The vaccines of the present invention stimulate strong Th1-type cytokine production

21. Experiments have been performed to investigate the effect of QS21/3D-MPL on the stimulation of IFN-γ (Th1-type cytokine), and the data is presented in the patent specification (page 9, 1.4 results). This data shows that the combination of the two adjuvants is capable of acting in a synergistic fashion in the induction of high levels of IFN-production

22. CTL responses are normally associated with strong Th1-type responses, thus CTL's can be considered to be a subset of TH1-type responses. As discussed above, HIV-specific CTL are thought to be correlates for protection, it may also be that other Th1-type effector components play a role in protection in combination with the CTL. As QS21 and 3D-MPL containing vaccines are capable of inducing both CTL, and other Th1-type responses (as disclosed in the patent application specification), it is expected that the crucial mixture CTL/Th1 effector components will be generated by vaccination with HIV vaccines containing QS21 and 3D-MPL.

Conclusions

23. In conclusion, vaccines of the present invention, namely combinations of 3D-MPL and QS21, together with HIV antigen, have been shown to have efficacy in one

of the best animal models currently available for the investigation of potential prophylactic HIV vaccines. Vaccines of the present invention, thus, provide a viable and potentially successful anti-HIV vaccine suitable for human use.

24. I declare that all statements made herein based on my own knowledge are true and that all statements based on information and belief are believed to be true; and further that the statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the above application or any patent issued therefrom.

Date: April 6th, 1998 Gerald Voss, Ph.D.

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Mouse model data

Table 1, *Vaccine formulations*

Each group consisting of 8 mice vaccinated twice and samples taken 7 days post second vaccination (V2).

Group	***Antigen***	***Adjuvant formulation***
1	-	SB62, 3D-MPL (5μg), QS21 (5μg)
2	gp120 (5μg)	3D-MPL (5μg), QS21 (5μg)
3	gp120 (5μg)	Alum (50μg)

NB, SB62 is an oil in water emulsion comprising squalene, α-tocopherol, and TWEEN80™

Table 2, *Serological results*

Group	***ELISA titres (GMT)***	***IgG1 %***	***IgG2a %***	***IgG2b %***
1	0	0	0	0
2	45910	22	48	30
3	874	100	0	0

Table 3, *Summary table of results in the murine model*

Group	***Humoral Immunity***		***Cellular Immunity***					
			Lymph nodes			*Spleen*		
	ELISA	*IgG2a*	*Prolif*	*IL-2*	*CTL*	*Prolif*	*IL-2*	*CTL*
1	0	ND	0	0	0	0	0	0
2	++	++	++	+	++	+++	+	++
3	0	0	++	+/-	0	+	+	0

Figure 1. *CD8+ CTL detected in Popliteal lymph node cells 7 days post V1/*

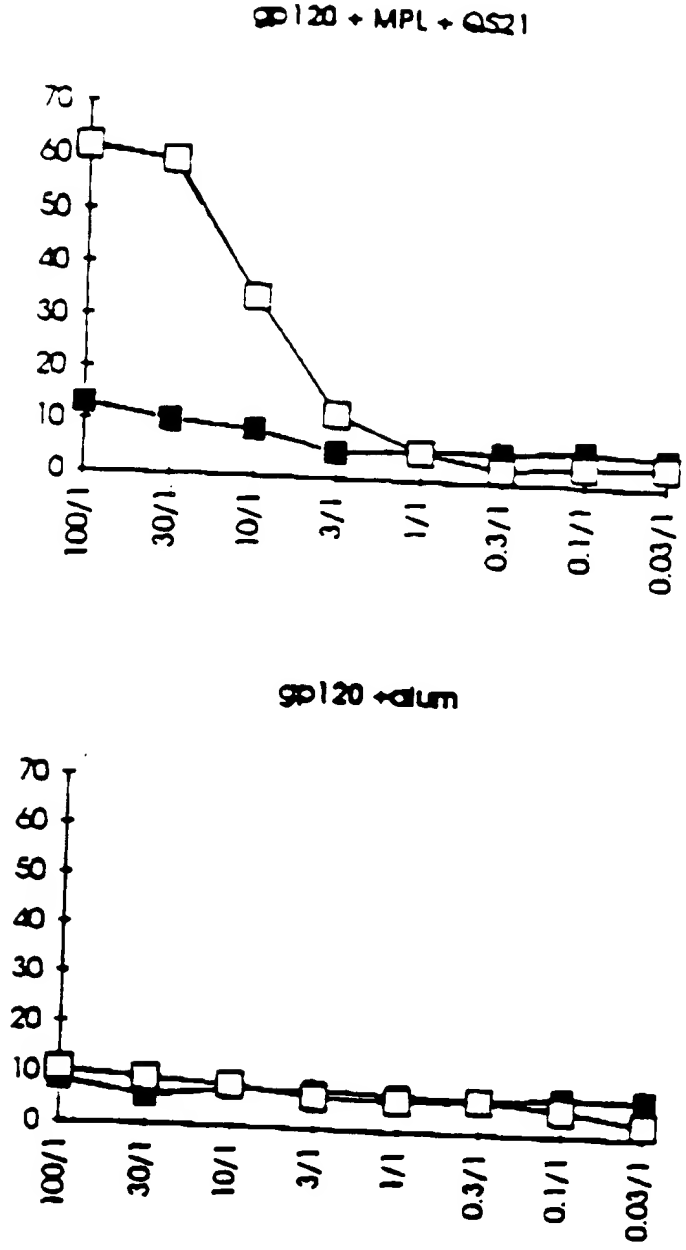
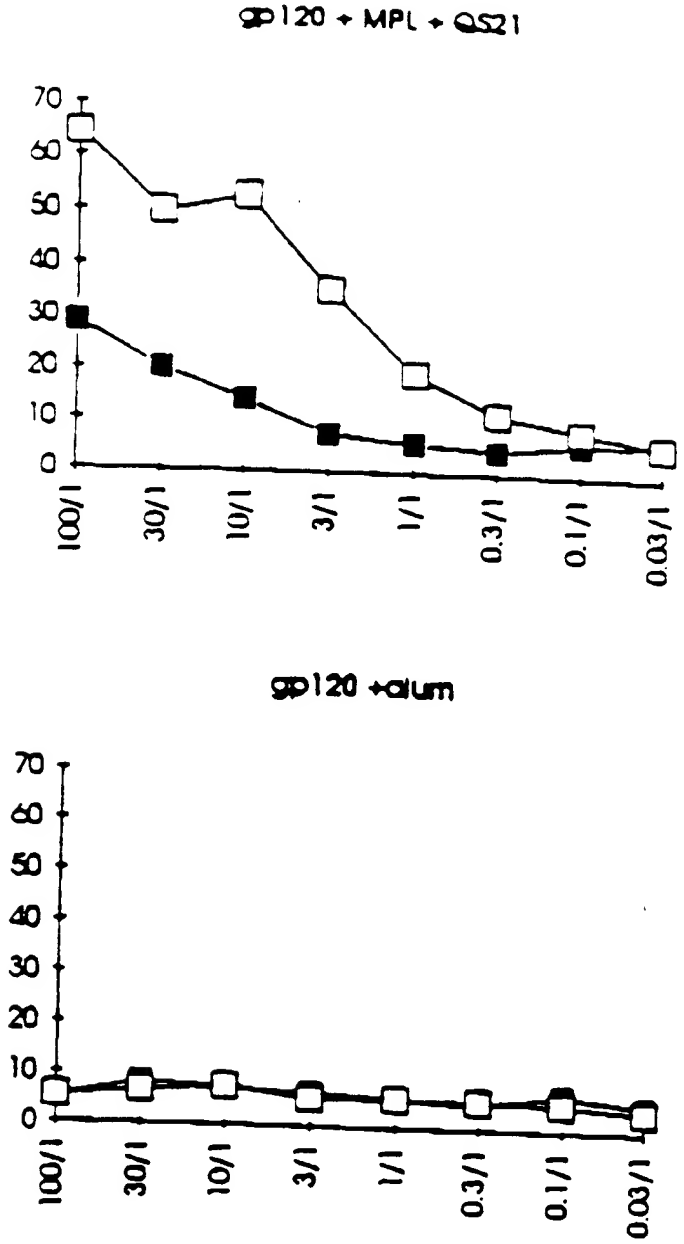


Figure 2. *CD8+ CTL detected in spleen cells 7 days post VII*



Rhesus monkey data

Rhesus monkeys were vaccinated at 0,1,3,12,19 months and challenged (28 days post V5) with homologous virus strain $SHIV_{W6.1D}$.

Table 4, *Vaccine formulations*

Group	***Antigen***	***Adjuvant formulation***
A	gp120 (100μg)	QS21 (50μg), 3D-MPL (50μg).
Control	-	-

NB, Vaccinations number 4 and 5 also contained small unilamenar liposomes. In house data shows that the addition of the liposomes does not significantly affect the immunological responses observed with after vaccination compared to 3D-MPL/QS21 alone. The function of this addition is to reduce reactogenicity and to prolong stability of QS21 during long-term storage.

Table 5, *Serology results post V2 and V3.*

The results presented constitute anti-gp120 titres as measured by ELISA and reciprocal dilutions of the highest dilution of serum to neutralise virus growth in vitro (VN).

Group	***Animal***	***2 weeks post V2***		***2 weeks post V3***	
		anti-gp120	*VN*	*anti-gp120*	*VN*
A	*9150*	59310	400	45400	1600
	9175	10227	100	14216	400
	9208	16393	200	21900	400
	9214	5788	100	9595	200
	mean (+/- sem)	22930 (+/- 12320)	200 (+/-70)	22778 (+/- 7957)	650 (+/-320)

Table 6, *Summary table of Rhesus monkey serology at time of viral challenge and outcome of challenge.*

Group	***Animal***	***Serology***		***Outcome to homologous challenge***
		anti-gp120	VN	
A	9150	25600	320	Protected
	9175	51200	640	Protected
	9208	25600	640	Infected
	9218	25600	320	Infected
Control	AA002	<20	40	Infected
	J040	<20	40	Infected
	L146	<20	<40	Infected
	Y005	<20	40	Infected